# Optofluidic microscopy

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**Abstract:** This research aims to integrate optical microscopy with microfluidic systems and create a device which is potentially capable of achieving sub wavelength resolution. This paper reports on our initial results of this optofluidic microscope (OFM).


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## 1. Introduction

Recent developments in the field of microfludics have been rapid and broad ranging. We have witnessed the development of large-scale integration of microfluidic circuits [1], and numerous applications of microfluidics in life science research [2]. Optical microscopy is presently employed in microfluidic research as a technique to study fundamental microscale flow physics as well as biological targets and processes conducted within these integrated microfluidic systems. At present the optical analysis units used with these microfluidic devices tend to be bulky and not particularly amenable to portability. Therefore, we have initiated an idea of integrating the optical imagers directly onto microfluidic circuits to create a very compact optical imager-on-a-chip.

Such an OFM system has a wide range of applications. It can be used to perform rapid screening of white blood cells in clinical blood specimens and to distinguish cancer cells from normal cells in flow cytometry analysis. An OFM system will also be very useful in those areas where a portable and rugged optical measurement system is desired. In addition, by making use of nanofabrication technology and the evanescent wave optics [3], we aim to achieve optical resolution on the order of 10's of nanometers. A sub-wavelength resolution system such as this one could be used to optically image very small bio-entities, such as bacteria and viruses.

## 2. Methods

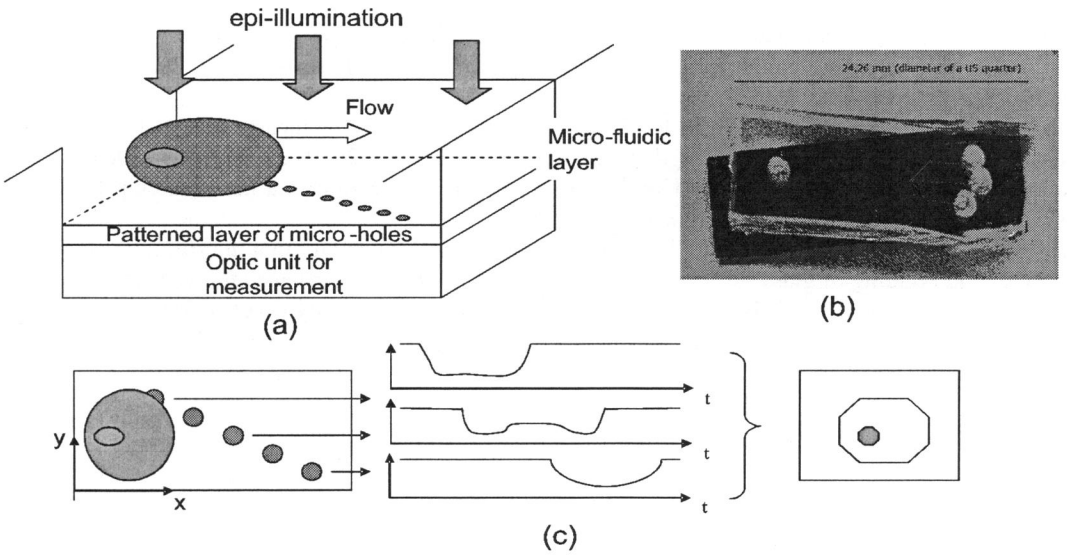


Figure 1: Optofluidic microscope setup: (a) layout schematic; (b) photograph (scale b ar=24.26 mm); (c) Schematic of Image capturing process;

Figure 1 shows the setup schematically. The microfluidic layer is fabricated by soft lithography and is bonded onto the detection layer. As shown in Fig. 1(a), the targeted object is made to flow through the microfluidic channel at a constant velocity using either electrokinetic control [4] or pressure driven flow and confined to the center of the imager through upstream focusing. In its current arrangement the device is illuminated from the top. The detection layer consists of a slide of gold-coated glass (passivated with PMMA on the upper surface to ensure electrical isolation from the fluidic system). The gold layer is sufficiently thick, so that the transmission of an incident light field is negligible. A slanted hole array is etched on this film, thus generating a line of light 'receivers'. Demonstrated in Fig. 1(c), the time evolution of the light power penetrating through each sub-micron hole is dependent on the profile of the target as well as its optical properties. The transmission may be monitored by simply attaching a linear CCD or photodiode array directly underneath the detection layer. As seen in Figure 1 (c) the slanted hole array fills the entire width of the microfluidic channel. The spacing of the holes is designed to be the same as the line scan sensor (e.g. Dalsa tall pixel sensor IL-C6-2048), so that the transmission through each hole will uniquely map to one single CCD pixel. Assuming that the flow of the target object within the channel is at a constant velocity and that the object is un-deviated during this transportation, the shape or absorption profile of the object can be deduced from the time traces of the hole transmissions as measured by the CCD array.

The pixel resolution in y-direction (perpendicular to flow direction as shown in Fig 1 (c)) depends on the spacing of adjacent holes in this direction. In the case where the channel width is 20 microns, the y-direction pixel size would be 500 nm if there are 40 holes across the channel. That is to say,

$$\text{pixel size in y direction} = \frac{\text{channel width}}{\text{total number of holes}} \qquad (1)$$

The pixel resolution in the x-direction is determined by the acquisition rate of the optical measurement unit and the flowing speed of the target. The pixel size in x-direction is equal to target moving speed $u$ times the pixel acquisition time $\Delta\tau$.

$$\text{Pixel size in x direction} = u \cdot \Delta\tau \qquad (2)$$

For example, if the target flow speed is 1.0 millimeter per second, and the detector's reading rate is 10 KHz, the pixel size in x direction would be equal to 100 nanometers.

Sub-wavelength resolution can be achieved in an OFM device by simply spacing the adjacent holes in the y-direction at the desired resolution limit. As the holes are well separated in x-direction by 10's of microns, their transmission contributions will be distinguishable from each other. The state of the art nanofabrication technology enables the creation of etching patterns with resolution of 10's of nanometer. Therefore, it should be possible to create OFM devices with resolution of sub 100 nanometers

We employed standard micro-fabrication techniques to create our prototype OFM device. Gold film of thickness about 1000 A was thermally coated on a thin glass slide, and then 200 nm thick electron beam resist, Polymethyl-methacrylate (PMMA) was spin coated onto the gold film. The hole array was patterned onto the PMMA by e-beam lithography. The PMMA layer was then developed and the hole array pattern was etched onto the gold film in the final development process. The x-direction spacing between the holes was 12.90 microns, while the y-direction spacing was 1.25 microns.

The microfluidic channels are made from the soft lithography technology based upon poly(dimethylsiloxane) (PDMS). The channel width was 50 microns in the initial experiment; the achievable channel width was limited only by the resolution of the soft lithography process and its choice is dependent on specific applications. The PDMS chip was treated with oxygen plasma so that the resulting channel will be hydrophilic and to ensure that the PDMS chip will seal well with the detection layer. Placement of the PDMS chip onto the detection layer was performed under a microscope to ensure optimal placement of the channel with respect to the hole array.

### 3. Results

Figure 1(b) is a photograph of our initial OFM device; the scale bar is equal to the diameter of a US quarter. In the initial experiment, the OFM device was not directly mounted onto a CCD camera, instead the transmissions through the hole array were imaged and acquired through an imaging inverted microscope (Olympus IX-71) and a micropix 1394 camera (C-1024). The test objects were flowed through the channel; the flow was driven by pressure gradient. In our demonstration, the test objects were latex micro-spheres. The temporal signal changes at each pixel are shown in Figure 2. These temporal changes may be analyzed and the target's geometric profile can be reconstructed.

This preliminary study demonstrates that the OFM imaging method is feasible. More importantly, the technology employed in the demonstration is readily scalable. An OFM device with sub-wavelength resolution is well within our means to fabricate. The advantages of the optofluidic microscope include high achievable resolution, low cost, compactness, small sample volume, ease of view finding and high throughput.

In addition to the above mentioned 'spatial-encoding' approach used in our prototype OFM device, we may also use spectral encoding approaches to create other types of OFM imaging system. One such example of spectral encoding based imaging system consists of simply embedding semiconductor quantum dots with different fluorescence emission spectra in a two-dimensional hole matrix. By observing the time changing emission spectra from the matrix, we can deduce the profile of the test object that is flowing across detection matrix unit. Research into this encoding scheme is in progress.

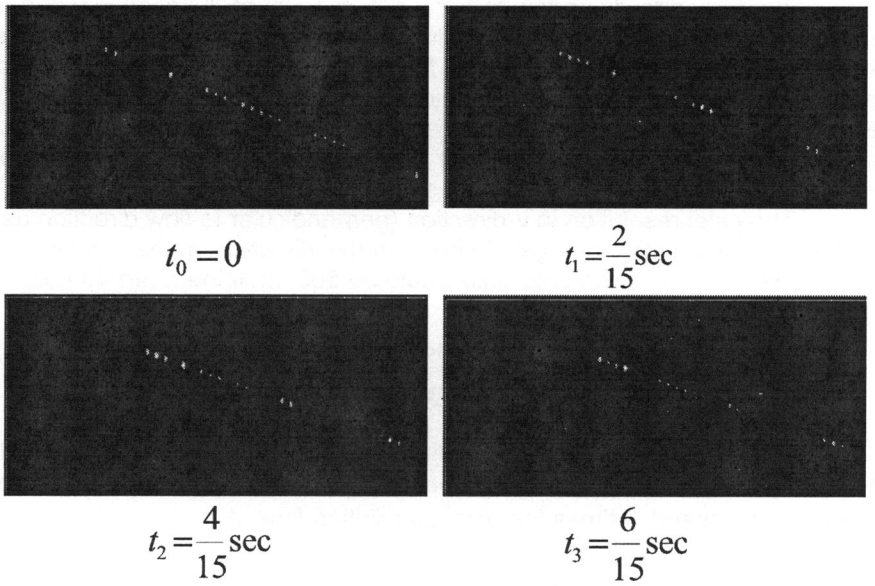


Fig 2: Time trace of the transmission taken by micropix C-1024. Hole spacing is 13um

### 4. Conclusion

We demonstrated a new type of imaging microscope that is dramatically compact (in principle, the device can be fabricated to be of the size of matchbox), is capable of high throughput processing, and is easy to implement at a mass production level. This novel imaging microscope, based on the creative use of optics and microfluidic technology, can dramatically increase the application range of microscopy and simplify existing biomedicine analysis procedures.

### 5. References

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